**Electronic Supplementary Information**

# Poly-lysine supported cross-linked enzyme aggregates with efficient enzymatic activity and high operational stability

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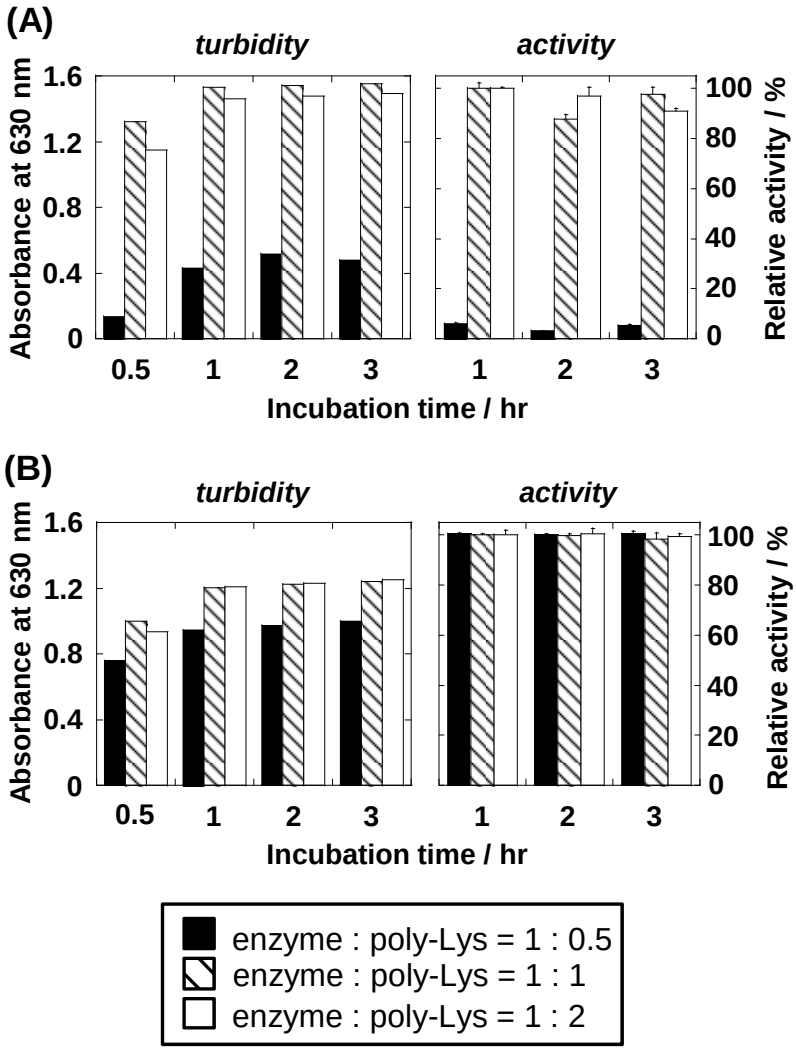


**Fig. S1** Effect of poly-Lys on CLEA formation and hydrolytic activity for CT (A) and SBc (B). The cross-linking reaction was conducted at 4°C with the mixture of PA (4%, v/v) and GA (0.25%, v/v). The turbidity of the reaction mixture was measured by the absorbance at 630 nm. Hydrolytic reactions were conducted in 20 mM PB (pH 7.5) at 30°C. Substrate: GPNA (1 mM) for CT-CLEA; Suc-AAPF-*p*NA (200 μM) for SBc-CLEA. The graph shows the mean ± standard error for at least three experiments.

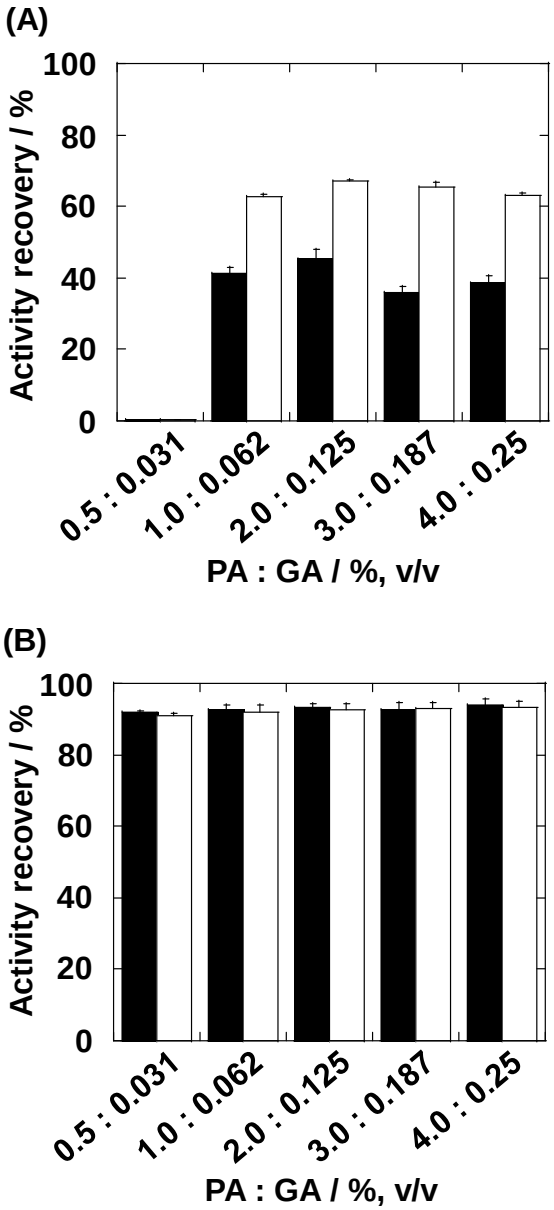


**Fig. S2** Enzymatic activities of CT-CLEA (A) and SBc-CLEA (B) prepared using different concentrations of cross-linker (PA and GA). The ratio of PA to GA was constantly 16:1 (v/v). All assays were performed in 20 mM PB (pH 7.5) at 30°C (closed bars) and 50°C (open bars). Concentrations of free CT and free SBc were 50 and 2 μg $mL^{-1}$, respectively. Other experimental conditions were identical to those in Fig. S1. The results are presented as the activity recovery to free proteases at 30°C. The graph shows the mean ± standard error for at least three experiments.

**Table S1.** Kinetic parameters for the hydrolysis of GPNA by CT-CLEA at pH 7.5 or pH 5.5[a]

| | pH 7.5 | | | pH 5.5 | | |
|---|---|---|---|---|---|---|
| PA:GA %:% (v/v) | $K_m$ (mM) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($M^{-1}$ $min^{-1}$) | $K_m$ (mM) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($M^{-1}$ $min^{-1}$) |
| 1.0:0.062 | 1.15 ± 0.13 | 0.49 ± 0.03 | 426 | 0.56 ± 0.05 | 0.40 ± 0.02 | 714 |
| 2.0:0.125 | 0.89 ± 0.05 | 0.53 ± 0.02 | 596 | 0.47 ± 0.01 | 0.49 ± 0.003 | 1043 |
| 3.0:0.187 | 1.40 ± 0.18 | 0.80 ± 0.06 | 571 | 0.56 ± 0.05 | 0.65 ± 0.02 | 1161 |
| 4.0:0.25 | 0.90 ± 0.05 | 0.46 ± 0.01 | 511 | 0.43 ± 0.02 | 0.46 ± 0.01 | 1070 |
| In solution[b] | 1.57 ± 0.29 | 1.02 ± 0.09 | 650 | 3.64 ± 1.37 | 0.71 ± 0.19 | 195 |

Values represent averages from results obtained from 2–3 independent experiments. [a] Reactions were conducted at 30°C. [b] Concentration of CT was 50 μg $mL^{-1}$.

**Table S2** Kinetic parameters for the hydrolysis of Suc-AAPF-*p*NA by SBc-CLEA at pH 7.5 or pH 5.5[a]

| | pH 7.5 | | | pH 5.5 | | |
|---|---|---|---|---|---|---|
| PA:GA %:%, (v/v) | $K_m$ (mM) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($M^{-1}$ $min^{-1}$) | $K_m$ (mM) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($M^{-1}$ $min^{-1}$) |
| 0.5:0.031 | 2.36 ± 1.14 | 28 ± 0.1 | $12 \times 10^3$ | 0.54 ± 0.03 | 6 ± 0.2 | $11 \times 10^3$ |
| 4.0:0.25 | 2.54 ± 0.26 | 34 ± 3 | $13 \times 10^3$ | 0.89 ± 0.09 | 9 ± 0.6 | $10 \times 10^3$ |
| In solution[b] | 3.36 ± 0.25 | 17944 ± 1143 | $5341 \times 10^3$ | 0.63 ± 0.03 | 582 ± 13 | $923 \times 10^3$ |

Values represent averages of results obtained from 2–3 independent experiments. [a] Reactions were conducted at 30°C. [b] Concentration of SBc was 2 μg $mL^{-1}$.